

RESEARCH LETTER

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Key Points:

- Nitrite addition stimulated nitrite oxidation in both oxic and anoxic waters
- Natural assemblages of marine nitrite-oxidizing bacteria have high affinity for nitrite
- Addition of oxygen at μM -level inhibited nitrite oxidation in oxygen depleted waters

Supporting Information:

- Supporting Information S1

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Dependence of nitrite oxidation on nitrite and oxygen in low-oxygen seawater

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Abstract Nitrite oxidation is an essential step in transformations of fixed nitrogen. The physiology of nitrite oxidizing bacteria (NOB) implies that the rates of nitrite oxidation should be controlled by concentration of their substrate, nitrite, and the terminal electron acceptor, oxygen. The sensitivities of nitrite oxidation to oxygen and nitrite concentrations were investigated using ^{15}N tracer incubations in the Eastern Tropical North Pacific. Nitrite stimulated nitrite oxidation under low in situ nitrite conditions, following Michaelis-Menten kinetics, indicating that nitrite was the limiting substrate. The nitrite half-saturation constant ($K_s = 0.254 \pm 0.161 \mu\text{M}$) was 1–3 orders of magnitude lower than in cultivated NOB, indicating higher affinity of marine NOB for nitrite. The highest rates of nitrite oxidation were measured in the oxygen depleted zone (ODZ), and were partially inhibited by additions of oxygen. This oxygen sensitivity suggests that ODZ specialist NOB, adapted to low-oxygen conditions, are responsible for apparently anaerobic nitrite oxidation.

Plain Language Summary Nitrite is a key intermediate in the biogeochemistry of low-oxygen marine environments, including the loss of fixed nitrogen as dinitrogen gas and nitrous oxide. Nitrate reduction to nitrite coupled to reoxidation of nitrite to nitrate has been proposed as a cycle that can preserve bioavailable nitrogen in oxygen minimum zones. This cycle implies that nitrite oxidation occurs in the absence, or near absence, of oxygen. Nitrite oxidation is considered to be an obligately aerobic process, although it has been reported from anoxic waters. Here we report on the regulation of nitrite oxidation by oxygen and nitrite in natural assemblages from the oxygen minimum zone of the Eastern Tropical North Pacific. We show that natural assemblages have very high affinity for nitrite and that oxygen actually inhibits nitrite oxidation in anoxic samples. These findings have implications for the marine nitrogen budget now and in future scenarios of changing ocean conditions.

1. Introduction

Nitrogen (N) is an essential nutrient for organisms on Earth. N limits primary production in many parts of the ocean and thus plays an important role in controlling CO_2 uptake by the ocean. All N transformations, including the net loss of fixed N, occur in oxygen minimum zones (OMZs) due to the coexistence of oxic and anoxic environments. OMZs are characterized by a strong O_2 gradient (oxycline) overlying a layer of high nitrite (NO_2^-) concentration coinciding with an oxygen-depleted zone (ODZ) where O_2 concentration is low enough to induce anaerobic processes. OMZs are “hot spots” of N loss [Codispoti, 1995; Gruber and Sarmiento, 1997; Ulloa et al., 2012] via denitrification and anammox [Ward et al., 2009; Lam and Kuypers, 2011; Babbitt et al., 2014]. Three major OMZs, the Eastern Tropical North Pacific (ETNP), the Eastern Tropical South Pacific (ETSP), and the Arabian Sea, all together contain less than 1% of global seawater in volume but are responsible for up to 30% of N loss in the ocean [Codispoti et al., 2001]. The global expansion of OMZs that is predicted to result from global warming stresses the importance of understanding the N budget in these regions [Stramma et al., 2008].

In addition to N loss transformations, the upper boundaries of the ODZ layers are also sites of intense N cycling, linking aerobic (e.g., nitrification) and anaerobic processes across the oxycline. Even in the core of the ODZ, where O_2 is undetectable, nitrifying microbes have been detected [Lam et al., 2009; Newell et al., 2011; Beman et al., 2012; Peng et al., 2013]. The first step of nitrification, ammonium oxidation, could be detected at O_2 concentration of 6 nM [Bristow et al., 2016] but has not been detected at apparently anoxic ODZ depths [Lam et al., 2009; Newell et al., 2011; Peng et al., 2016]. There are multiple reports of NO_2^- oxidation at depths where O_2 is apparently absent [Lipschultz et al., 1990; Füssel et al., 2012; Kalvelage et al., 2013; Peng et al., 2016]. This means that NO_2^- is not only being reduced to gaseous N in OMZs, but is also being

oxidized into bioavailable nitrate (NO_3^-), even in the absence of O_2 . Anaerobic NO_2^- oxidation to NO_3^- has not been detected in cultivated nitrite oxidizing bacteria (NOB), although some anaerobic metabolism (e.g., NO_3^- reduction) has been demonstrated for the NOB genera, *Nitrobacter* [Freitag *et al.*, 1987] and *Nitrospira* [Koch *et al.*, 2015]. *Nitrospira*, in the newly proposed *Nitrospinae* phylum [Lücker *et al.*, 2013], has been identified as the dominant NOB genus in ODZs [Beman *et al.*, 2013; Levipan *et al.*, 2014; Ganesh *et al.*, 2015] and thus may be the main contributor to apparently anaerobic NO_2^- oxidation. Observations of apparently anaerobic NO_2^- oxidation in the environment are difficult to reconcile with documented metabolic capabilities of known NOB.

To decipher the puzzle of apparently anaerobic NO_2^- oxidation, the controlling factors of the reaction need to be examined. As in other enzyme-catalyzed reactions, the apparent volumetric rate of NO_2^- oxidation is determined by substrate concentration, the catalytic capacity reflected by substrate affinity and the population size of NOB (equivalent to enzyme concentration). The substrate (NO_2^-) dependence of marine NO_2^- oxidation has not been determined, and a very limited number of studies have analyzed the effect of O_2 on the reaction. Previous studies conducted in low-oxygen seawater reported that O_2 had consistently positive effect on NO_2^- oxidation in samples from different depths [Bristow *et al.*, 2016] or had different effects [Kalvelage *et al.*, 2013; Bristow *et al.*, 2017]. Different depths might have different population sizes or types of NOB [Füssel *et al.*, 2012], which can also influence the apparent NO_2^- oxidation kinetics. In order to control for the population size and type of NOB, it is necessary to examine the effect of NO_2^- and O_2 concentrations on NO_2^- oxidation rates within samples from the same depth instead of combining samples from different depths. In this study, we used ^{15}N tracer incubation experiments to investigate the depth distribution of NO_2^- oxidation and its dependence on availabilities of NO_2^- and O_2 in the OMZ of the ETNP.

2. Materials and Methods

2.1. Experimental Site, Sample Collection, and Incubation

Seawater was collected from six stations (coastal stations PS1 and 8; offshore stations PS2, PS3, 11, and 14; Figure S1 in the supporting information) in the ETNP in April 2016 on board R/V *Ronald H. Brown* (Cruise ID: RB-16-03). NO_2^- oxidation rate profiles were obtained from stations PS1 and PS2. NO_2^- kinetics experiments were performed at stations PS1, 11, and PS3. Samples from stations 8 and 14 were used for O_2 kinetics experiments.

Water samples from each depth were collected into five 60 mL serum bottles using 10 L Niskin bottles on a rosette with a conductivity-temperature-depth profiler (CTD) or using a pump profiling system (PPS). In situ O_2 concentration (detection limit 2.1 μM), temperature, pressure, and salinity were recorded during each CTD or PPS cast. NO_2^- and NO_3^- concentrations were measured by standard spectrophotometric methods onboard [United Nations Educational, Scientific and Cultural Organization, 1994]. For sampling from Niskins from the CTD casts, serum bottles were filled after overflowing three times to minimize O_2 contamination and were sealed with rubber septa and aluminum rings immediately after filling. For PPS sampling, bottles were sealed with septa while submerged under seawater pumped from selected depth to avoid oxygenation from the atmosphere. A helium headspace (3 to 10 mL) was introduced into the bottle to facilitate mixing of tracers and O_2 adjustment (see below). ^{15}N - NO_2^- tracer ($^{15}\text{N}/(^{14}\text{N} + ^{15}\text{N}) = 99$ atom %) was injected into all five bottles from the same depth to reach a final concentration of 0.4 μM , except for the NO_2^- kinetics experiments in which the final ^{15}N - NO_2^- tracer concentration varied: 0.05, 0.1, 0.15, 0.2, 0.4, and 1 μM . For the O_2 kinetics experiments, ambient seawater was vigorously shaken and exposed to air to reach O_2 saturation. Then 0 to 5.0 mL of O_2 saturated seawater was added into serum bottles to achieve final O_2 concentrations of 0.03, 0.30, 0.70, 1.39, 2.74, and 6.84 μM in seawater. O_2 concentration was calculated assuming equilibrium between the water and the gas phases in serum bottles at the incubation temperature (20°C) [Garcia and Gordon, 1992]. A set of five bottles incubated in time series (one bottle as t_0 , two bottles as t_1 , and two as t_2) was used to determine each single rate. Incubations varied from 15 to 24 h and were carried out in controlled temperature rooms ($\pm 3^\circ\text{C}$ of in situ temperatures). Incubations were terminated by adding 0.1 mL saturated mercuric chloride (HgCl_2) into each serum bottle. Samples were stored at room temperature (18–22°C) in the dark for less than 8 months until analysis in the laboratory.

2.2. Isotope Measurement and Rate Determination

To determine the rate of nitrite oxidation to nitrate, the isotopic composition of NO_3^- in each bottle was measured by the denitrifier method [Sigman *et al.*, 2001; Weigand *et al.*, 2016]. Briefly, *Pseudomonas chlororaphis* (ATCC® 43928™) was cultured and concentrated 7 to 10 times by centrifugation in $\text{NO}_3^-/\text{NO}_2^-$ -free medium. To avoid interference of nitrite when using the denitrifier method, NO_2^- was removed from samples with sulfamic acid [Granger and Sigman, 2009]. Sulfamic acid treated sample (0.4 mL) was aliquoted into each 20 mL vial with concentrated bacteria (1 mL). NO_3^- in the vials was converted into N_2O , which was then measured on a mass spectrometer (Delta V^{plus}) to determine the nitrogen isotope composition ($\sigma^{15}\text{N}$) [McIlvin and Casciotti, 2011].

Because no significant change in nitrate concentrations was observed, and the ^{15}N content in nitrate samples are low, $[\text{NO}_3^-] = [^{14}\text{NO}_3^-] + [^{15}\text{NO}_3^-] \approx [^{14}\text{NO}_3^-]$. Because it is a tracer experiment, the effect of biological fractionation during the incubation experiments is negligible. The nitrite oxidation rate is calculated according to equation (1). V is the nitrite oxidation rate, F is the fraction label of nitrite, and $\frac{d[^{15}\text{NO}_3^-]/[^{14}\text{NO}_3^-]}{dt}$ is the rate of change of nitrate $^{15}\text{N}/^{14}\text{N}$, which was determined by the linear regression of time course experiments ($n = 5$). The error bar for each NO_2^- oxidation rate represents the standard error of the slope. The small fraction of $^{15}\text{N}-\text{NO}_3^-$ contamination in the $^{15}\text{N}-\text{NO}_2^-$ tracer [Peng *et al.*, 2015] did not influence the rate calculation because the rate of change in $^{15}\text{N}-\text{NO}_3^-$ over the time course, rather than the absolute value of $^{15}\text{N}-\text{NO}_3^-$ at the endpoint, was used to determine the rate.

$$V = \frac{1}{F} \times [\text{NO}_3^-] \times \frac{d[^{15}\text{NO}_3^-]/[^{14}\text{NO}_3^-]}{dt} \quad (1)$$

2.3. Kinetics Models and Rate Estimation

We used the Michaelis-Menten model ([Monod, 1942], equation (2)) to evaluate the effect of NO_2^- concentration on NO_2^- oxidation rate. V_m is the potential maximum rate of NO_2^- oxidation when $[\text{NO}_2^-]$ is not limiting. K_s is the half-saturation constant, the $[\text{NO}_2^-]$ at which the NO_2^- oxidation rate (V) equals half of V_m . Equation (3) was used to characterize the inhibition effect of O_2 on NO_2^- oxidation. In equation (3), m is the potential maximum inhibition by O_2 and n , analogous to half-saturation constant, is the half-inhibition constant, i.e., the concentration of O_2 that caused half of the potential maximum inhibition. The rates of NO_2^- oxidation were normalized to the largest measured rate, which is the rate measured under in situ $[\text{O}_2]$ ($V_{\text{in situ O}_2}$). The curve fitting tool in Matlab was used to fit the two equations to the measured NO_2^- oxidation rates and NO_2^- or O_2 concentrations.

$$V = V_m \times \frac{[\text{NO}_2^-]}{[\text{NO}_2^-] + K_s} \quad (2)$$

$$\frac{V}{V_{\text{in situ O}_2}} = 1 - m \times \frac{[\text{O}_2]}{[\text{O}_2] + n} \quad (3)$$

The Michaelis-Menten equation (2) was used to predict the potential maximum rates and the in situ rates of NO_2^- oxidation. Assuming a universal catalytic capacity of enzymes (same K_s), the potential maximum rate (V_m) at each depth was predicted by introducing the measured NO_2^- oxidation rate (V), its corresponding total $[\text{NO}_2^-]$ (in situ plus tracer addition), and the average half-saturation constant ($K_s = 0.254 \mu\text{M}$) into equation (2) (Figure 1). The predicted in situ rate was obtained by introducing V_m , in situ $[\text{NO}_2^-]$ and K_s into equation (2).

3. Results

3.1. Depth Distribution of NO_2^- Oxidation

NO_2^- oxidation rates were determined at four or five depths at two stations. Depths were chosen to represent features: the upper oxycline, the top of the ODZ, the core of the ODZ, and the lower oxycline. Highest measured NO_2^- oxidation rates at both stations were detected at ODZ depths (where oxygen concentrations were below detection using the Seabird sensor on the CTD; Figure 1). At station PS1, the highest measured

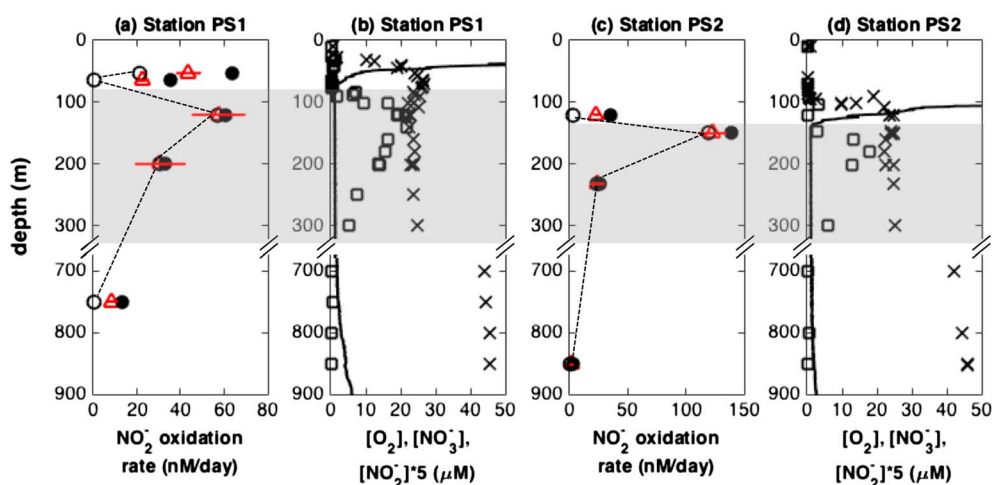


Figure 1. Profiles of NO_2^- oxidation rates and O_2 , NO_2^- and NO_3^- concentrations. Shaded areas indicate ODZs ($[\text{O}_2] < \text{CTD detection limit}$). (a, c) Measured rate (red triangle), predicted in situ rate (open circle connected by dashed lines), and predicted maximum rate (filled circle). (b, d) $[\text{O}_2]$ (solid line), $[\text{NO}_2^-]$ (open square), and $[\text{NO}_3^-]$ (cross). Measured rates = slope of linear regression of five independent time course bottles. Error bars = standard error of the regression coefficient.

rate was $57 \pm 11.8 \text{ nM d}^{-1}$ at the shallower depth in the core of ODZ (120 m). The measured rate ($31 \pm 11.1 \text{ nM d}^{-1}$) at the deeper ODZ depth (200 m) was not significantly different from measured rates at the two oxycline depths. At station PS2, the highest measured rate was $123 \pm 11.1 \text{ nM d}^{-1}$ in the top of the ODZ (150 m), which was almost 6 times of that at the oxycline depth. The lowest rates at both stations were detected in the lower oxycline.

3.2. Effect of NO_2^- on NO_2^- Oxidation Rate

NO_2^- oxidation kinetics were determined by manipulating the NO_2^- concentrations in replicate subsamples from the same Niskin bottle. The relationship between NO_2^- oxidation rates and NO_2^- concentrations in both oxic and anoxic samples with low in situ NO_2^- concentrations (0.1 or $0.05 \mu\text{M}$) followed the Michaelis-Menten relationship ([Monod, 1942]; equation (2)) with large r^2 values ($= 0.941$ and 0.989 , respectively), indicating that NO_2^- was the only limiting substrate in the reaction (Figures 2a and 2b). In other words, the in situ O_2 concentration was high enough for the reaction or was not a substrate of the reaction. The rates depend on the physiological characteristics of the NOB and the size of the NOB population, but the half-saturation constant does not depend on population size. The two half-saturation constants (average $K_s = 0.254 \pm 0.161 \mu\text{M}$) from two different stations with different in situ O_2 and NO_2^- concentrations were not significantly different, revealing that NOB from these samples had similar high substrate affinity. The NO_2^- oxidation rates increased with increasing NO_2^- concentrations but were not well represented by the Michaelis-Menten relationship when in situ NO_2^- concentration ($3.7 \mu\text{M}$) was much higher than the K_s (Figure 2c).

The relationship between NO_2^- oxidation rate and substrate concentration was also investigated by plotting NO_2^- oxidation rates measured in the depth profiles (nine depths at stations PS1 and PS2; Figure 1) against the NO_2^- concentration in each incubation (Figure S2). A Michaelis-Menten curve is not suitable to fit these data because they represent rates from samples with different population sizes. The relationship does not intersect zero, although the rate increases with increasing NO_2^- concentration in the oxycline samples, which reinforced the idea that NO_2^- was the limiting substrate of the reaction. NO_2^- oxidation rates at ODZ depths did not show NO_2^- dependence, however, which may imply differences in the size or characteristics of NOB populations in environments with different O_2 conditions.

3.3. Effect of O_2 Concentration on NO_2^- Oxidation Rate

O_2 kinetics were assayed in samples from the top of the ODZ, where O_2 concentration was below the CTD detection limit and the highest rates of NO_2^- oxidation occurred (Figure 1). Under in situ O_2 concentrations,

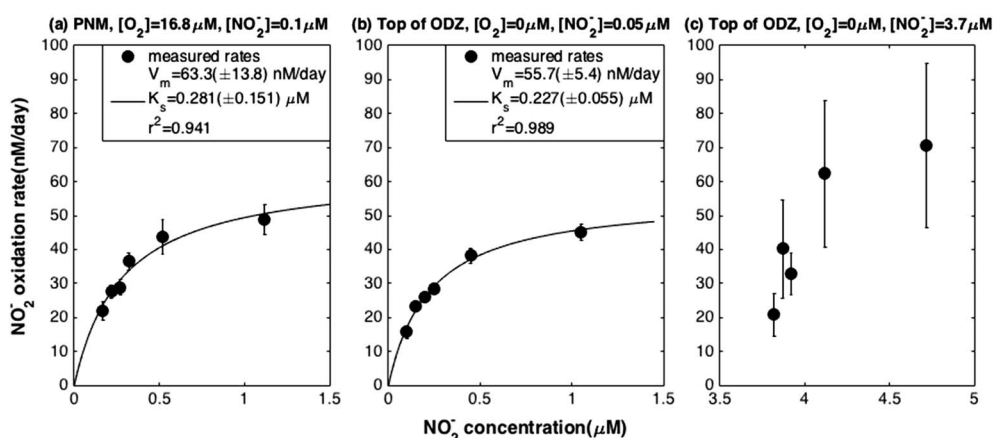


Figure 2. NO_2^- dependence of NO_2^- oxidation rates at (a) 53 m at station PS1, (b) 170 m at station PS3, and (c) 130 m at station 11. Measured rates = slope of linear regression of five independent time course bottles. Error bars = standard error of the regression coefficient. Michaelis-Menten equation was fitted to NO_2^- oxidation rates and NO_2^- concentrations in Figures 2a and 2b. Adjusted r^2 , coefficients (V_m and K_s) of the best fit and their 95% confidence intervals are shown in the figure. In situ feature, $[\text{O}_2]$ and $[\text{NO}_2^-]$ are shown for each figure. PNM = primary nitrite maximum.

nitrite oxidation rates were 143 ± 3.4 and $168 \pm 17.9 \text{ nM d}^{-1}$ at stations 8 and 14 with total NO_2^- concentrations of $0.43 \mu\text{M}$ (in situ $[\text{NO}_2^-] = 0.03 \mu\text{M}$) and $1.1 \mu\text{M}$ (in situ $[\text{NO}_2^-] = 0.7 \mu\text{M}$), respectively. The increased O_2 concentrations inhibited NO_2^- oxidation rates following an inhibition curve (Figure 3, equation (3)). At the two stations, the potential maximum inhibitions (m) by O_2 were 57.3% and 80.1%, respectively. The half-inhibition concentrations (n) of O_2 were 1.24 and $2.17 \mu\text{M}$, respectively.

4. Discussion

4.1. Effect of NO_2^- on NO_2^- Oxidation Rate

When controlled for population size, NO_2^- kinetics followed the Michaelis-Menten relationship. The half-saturation constant ($K_s = 0.254 \pm 0.161 \mu\text{M}$; average of two experiments, Figure 2) was 1–3 orders of magnitude

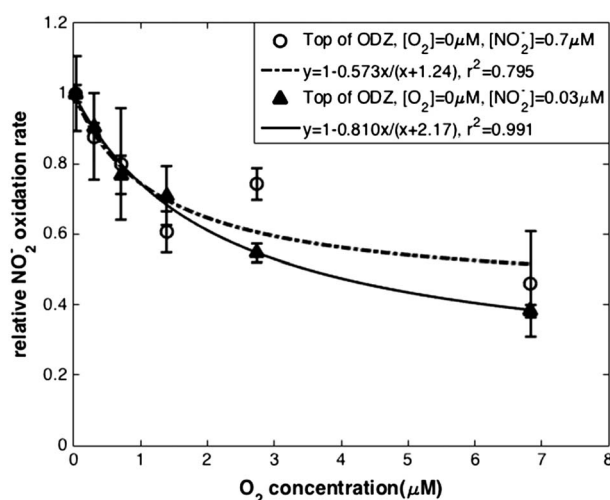


Figure 3. O_2 dependence of relative NO_2^- oxidation rates at the top of the ODZs. In situ $[\text{O}_2]$, $[\text{NO}_2^-]$, and the inhibition equations were shown for 89 m at station 8 (filled triangles) and 185 m at station 14 (open circles). Measured rates (V) = slope of linear regression of five independent time course bottles. Relative rates = $V/V_{\text{in situ } \text{O}_2}$. Error bars = standard error of the regression coefficient.

lower than that of pure cultures of three cultivated NOB genera (*Nitrobacter*, *Nitrotoga*, and *Nitrospira*; $K_s = 6\text{--}544 \mu\text{M}$) [Blackburne et al., 2007; Nowka et al., 2015; Ushiki et al., 2017]. $[\text{NO}_2^-]$ accumulates to μM levels in OMZs, suggesting that the K_s for NO_2^- might be even lower in regions with lower $[\text{NO}_2^-]$. The high affinity of marine NOB (presumably mainly *Nitrospina*-like) for NO_2^- suggests their adaptation to the NO_2^- -limited marine environment. This high affinity is analogous to the high affinity for NH_4^+ of the natural assemblages of NH_4^+ -oxidizing microbes [Horak et al., 2013; Newell et al., 2013; Peng et al., 2016], presumably mainly archaea, since high affinity has so far only been observed for the cultivated NH_4^+ -oxidizing archaeon *Nitrosopumilus maritimus* [Martens-Habbena et al., 2009].

4.2. Effect of O_2 Concentration on NO_2^- Oxidation Rate

Contrary to expectations for a metabolism that is assumed to require oxygen, increased $[O_2]$ consistently inhibited measured NO_2^- oxidation rates. Oxidants other than O_2 might also contribute to the NO_2^- oxidation, but all known marine NOB are obligate O_2 respirers and use electron transport chains that normally terminate with O_2 . Anammox bacteria also oxidize NO_2^- anaerobically to NO_3^- , during which about 1 mole of NO_2^- is oxidized to NO_3^- per 6 moles of NO_2^- reduced to N_2 [Oshiki *et al.*, 2016]. Anammox cannot be the major pathway of apparently anaerobic NO_2^- oxidation reported here, because measured rates of anammox in this region [Babbín, 2014] and in other OMZs [Hamersley *et al.*, 2007; Dalsgaard *et al.*, 2012; Babbín *et al.*, 2014] are much lower (by tenfold) than the NO_2^- oxidation rates reported here.

The decrease in NO_2^- oxidation rates with increasing O_2 concentration reported here was not a result of NO_2^- limitation. First, the total NO_2^- concentrations at the top of the ODZ at stations 8 and 14 are much higher than the K_s measured at nearby stations. Second, over the 24 h incubation time, a NO_2^- oxidation rate of 200 nM d^{-1} (larger than any measurement in this study) and a N_2 production rate of 60 nM N d^{-1} (larger than any measurement in this region [Babbín, 2014]) consume only $0.26 \text{ }\mu\text{M}$ of NO_2^- , which is much less than the total NO_2^- concentrations in these samples ($0.43 \text{ }\mu\text{M}$ and $1.1 \text{ }\mu\text{M}$). Therefore, the inhibition of NO_2^- oxidation by O_2 was the response of NOB assemblages to O_2 , independent of NO_2^- concentration.

These samples were collected from depths where O_2 was undetectable by the Seabird sensor ($<2.1 \text{ }\mu\text{M}$). Previous intercalibrations with the STOX sensor [Tiano *et al.*, 2014] indicated that the true O_2 concentration at the sample depths was likely below the STOX sensor detection limits of $\sim 10 \text{ nM}$. Unintended O_2 contamination during the sampling process likely occurred [Revsbech *et al.*, 2009], which could have caused uniform low-level contamination of all independent time course samples. This contamination has no effect on the shape of the inhibition curve, showing that NO_2^- oxidation decreased with increasing $[O_2]$ at μM level. It does influence, however, determination of the absolute $[O_2]$ where the inhibition starts. It is possible that nitrite oxidation is stimulated by increasing $[O_2]$ at nM level and saturated at $\sim 1 \text{ }\mu\text{M}$ as observed by Bristow *et al.* [2016] with a STOX sensor, but is inhibited by $[O_2]$ at μM level.

Sensitivity of NOB from ODZs to O_2 is consistent with adaptation to anoxic conditions, such that exposure to μM levels of O_2 might cause damage to their cells. This hypothesis is supported by the absence of classical reactive O_2 defense mechanisms in *Nitrospina gracilis* isolated from the surface ocean [Lücker *et al.*, 2013]. A previous study found that surface seawater and ODZs had different dominant *Nitrospina* OTUs [Beman *et al.*, 2013]. The present results suggest that ODZ NOB might be low O_2 specialists, in having lower O_2 tolerance than NOB from oxic environments. Additionally, a metagenomic study from the Arabian Sea found a novel lineage of the gene encoding nitrite oxidoreductase (*nrrA*) that clustered between anammox and *Nitrospina* sequences. This novel *nrrA* sequence was as abundant as *Nitrospina nrrA* at 600 m in the Arabian Sea ODZ [Lüke *et al.*, 2016]. A single-cell genomic study proposed a novel genus within the *Nitrospinae* phylum possessing not only the genetic repertoire for nitrite oxidation but also a nitrate reductase gene [Ngugi *et al.*, 2016]. Nitrite-dependent anaerobic methane oxidizers [Ettwig *et al.*, 2010; Haroon *et al.*, 2013] might oxidize nitrite by reverse reaction of their nitrate reductase enzymes. The nitrate reductase genes are homologous to nitrite oxidoreductase genes. Their contribution to nitrite oxidation in the ODZ, however, is likely to be small due to their low abundance (from undetectable to less than 0.1% of microbial communities) [Chronopoulou *et al.*, 2017; Padilla *et al.*, 2016]. These studies imply that other unidentified organisms, with functional genes homologous to those of anaerobes (i.e., anammox and denitrifiers) might be responsible for apparently anaerobic NO_2^- oxidation.

O_2 did not fully inhibit NO_2^- oxidation, i.e., $m < 100\%$, suggesting that parts of the NOB community in the upper ODZ might be similar to surface NOB living in oxic environments (i.e., oxyclines). The apparent percentage of surface NOB in the community varied among different stations. The stimulation of NO_2^- oxidation rate by increasing $[O_2]$ at nM level, which was observed in a recent study [Bristow *et al.*, 2016], might be explained by the higher affinity for oxygen of ODZ NOB and a larger proportion of the surface NOB in those samples from the upper oxycline and the ODZ boundary compared to the ODZ samples in this study. Bristow *et al.* [2016] detected a high rate (around 50 nM d^{-1}) of NO_2^- oxidation even when ambient O_2 was below the STOX sensor detection limit (10 nM), which might be evidence of the presence of ODZ specialist NOB. However, population size and community composition of NOB were not independently controlled in these

samples from three different depths, which might also affect the characterization of O_2 kinetics. It is analogous to plotting NO_2^- oxidation rates from multiple samples in this study (Figure S2); the combined data from different depths did not show the $[NO_2^-]$ dependence that was evident in experiments in which $[NO_2^-]$ and $[O_2]$ were independently varied within samples from the same depth.

4.3. NO_2^- Oxidation in ODZs and Its Significance

The NO_2^- oxidation rate in ODZs can never be fueled by O_2 contamination if there were no NOB in the seawater. The high rates detected in the ODZ in this and other studies [Lipschultz *et al.*, 1990; Peng *et al.*, 2015; Babbin *et al.*, 2017] together with molecular data [Füssel *et al.*, 2012; Beman *et al.*, 2013] directly support the idea that microbes with NO_2^- oxidation capacity were present in the anoxic seawater. Evidence in support of their in situ NO_2^- oxidation activities in ODZs is provided by model simulations [Buchwald *et al.*, 2015] and stable isotope measurements [Casciotti *et al.*, 2013; Gaye *et al.*, 2013; Peters *et al.*, 2016]. The isotopic composition of NO_2^- in the ODZ is relatively ^{15}N depleted, which is attributed to the inverse isotope effect during NO_2^- oxidation to NO_3^- [Casciotti, 2009].

To evaluate the effect of experimental conditions on measured rates, we used the kinetics data to predict in situ rates and potential maximum rates (V_m) (Figure 1). In the core of the ODZs, measured rates were not significantly different from V_m since the total NO_2^- concentrations were much higher than K_s . The predicted V_m was a proxy for the size of the NOB population, assuming similar substrate affinity of NOB involved in NO_2^- oxidation (similar K_s determined in NO_2^- kinetics experiments at two depths makes this a reasonable assumption). The predicted V_m varied among depths, indicating the necessity of controlling population size while characterizing NO_2^- or O_2 kinetics. The implied population size at the top of the ODZ was much larger than that at any other depth. The implied abundance of NOB at the top of the ODZs might be due to the relatively higher NO_2^- supply, and O_2 invasion events may enable the persistence of both ODZ specialist NOB and surface NOB. The inferred population size in the core of the ODZs was of the same magnitude as that in the oxycline. If NOB communities at ODZ depths consist only of surface NOB, which prefer oxic conditions, the puzzle of how they make a living and oxidize NO_2^- in the absence of O_2 must be solved. More likely, ODZ specialist NOB are dominant in the core of the ODZ where anoxia is more stable and the assemblage is more isolated from the oxic environment. However, NO_2^- oxidation rates in the core of the ODZ were higher than or similar to rates in surface seawater (Figure 1), implying that ODZ specialist NOB survived in the anoxic environment. One of the potential oxidants for NO_2^- oxidation in the ODZ is iodate; however, iodate alone is insufficient to support the measured rates [Babbin *et al.*, 2017]. Other potential oxidants that might contribute to oxidizing NO_2^- in these anoxic seawaters remain to be identified. Likewise the identity of potential ODZ specialist NOB and whether they are capable of conducting alternative anaerobic reactions (i.e., denitrification) await further exploration.

Regardless of the mechanism of NO_2^- oxidation in the ODZ, high NO_2^- oxidation rates detected in ODZs can be coupled with NO_3^- reduction to constitute an efficient $NO_2^- \longleftrightarrow NO_3^-$ cycle, which preserves bioavailable N in this intense N loss spot [Füssel *et al.*, 2012; Casciotti *et al.*, 2013; Peters *et al.*, 2016]. The $NO_2^- \longleftrightarrow NO_3^-$ cycle may also provide NH_4^+ to fuel anammox because NO_3^- is used as an electron acceptor to oxidize organic matter in the absence of oxygen, and thus liberates NH_4^+ . NO_3^- reduction to NO_2^- is a more widespread metabolic capacity of microbes than complete denitrification ($NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$), and NO_3^- reduction is the most important anaerobic respiration process in the low oxygen seawater, based on observed rates [Lipschultz *et al.*, 1990; Lam *et al.*, 2009]. Thus, NO_3^- reduction to NO_2^- would provide NH_4^+ in excess of that supplied by organic matter remineralization occurring during complete denitrification. A model that included NO_2^- oxidation in the ODZ successfully explained the formation of the secondary NO_2^- maximum [Babbin *et al.*, 2017]. The modeling exercise indicates the necessity of including the recycling between NO_2^- and NO_3^- in the anoxic environment in order to reevaluate the N budget in the ocean, especially the contribution of OMZs to N loss. This reevaluation is also important to predict the response of the N cycle and potential perturbation of the N budget under the expansion of OMZs.

References

- Babbin, A. R., R. G. Keil, A. H. Devol, and B. B. Ward (2014), Organic matter stoichiometry, flux, and oxygen control nitrogen loss in the ocean, *Science*, 344(6182), 406–408, doi:10.1126/science.1248364.
- Babbin A. R. (2014), Biogeochemical Controls on Fixed Nitrogen Loss Processes in the Marine Environment, PhD dissertation, Princeton Univ.

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- Babbin, A. R., B. D. Peters, C. W. Mordy, B. Widner, K. L. Casciotti, and B. B. Ward (2017), Multiple metabolisms constrain the anaerobic nitrite budget in the eastern tropical South Pacific, *Global Biogeochem. Cycles*, *31*, 258–271, doi:10.1002/2016GB005407.
- Beman, J. M., B. N. Popp, and S. E. Alford (2012), Quantification of ammonia oxidation rates and ammonia-oxidizing archaea and bacteria at high resolution in the Gulf of California and eastern tropical North Pacific Ocean, *Limnol. Oceanogr.*, *57*(3), 711–726, doi:10.4319/lo.2012.57.3.0711.
- Beman, J. M., J. L. Shih, and B. N. Popp (2013), Nitrite oxidation in the upper water column and oxygen minimum zone of the eastern tropical North Pacific Ocean, *ISME J.*, *7*(11), 2192–2205, doi:10.1038/ismej.2013.96.
- Blackburne, R., V. M. Vadivelu, Z. Yuan, and J. Keller (2007), Kinetic characterisation of an enriched *Nitrospira* culture with comparison to *Nitrobacter*, *Water Res.*, *41*(14), 3033–3042, doi:10.1016/j.watres.2007.01.043.
- Bristow, L. A., et al. (2016), Ammonium and nitrite oxidation at nanomolar oxygen concentrations in oxygen minimum zone waters, *Proc. Natl. Acad. Sci.*, *113*, doi:10.1073/pnas.1600359113.
- Bristow, L. A., et al. (2017), N_2 production rates limited by nitrite availability in the bay of Bengal oxygen minimum zone, *Nat. Geosci.*, *10*(1), 24–29, doi:10.1038/ngeo2847.
- Buchwald, C., A. E. Santoro, R. H. R. Stanley, and K. L. Casciotti (2015), Nitrogen cycling in the secondary nitrite maximum of the eastern tropical North Pacific off Costa Rica, *Global Biogeochem. Cycles*, *29*, 2061–2081, doi:10.1002/2015GB005187.
- Casciotti, K. L. (2009), Inverse kinetic isotope fractionation during bacterial nitrite oxidation, *Geochim. Cosmochim. Acta*, *73*(7), 2061–2076, doi:10.1016/j.gca.2008.12.022.
- Casciotti, K. L., C. Buchwald, and M. McIlvin (2013), Implications of nitrate and nitrite isotopic measurements for the mechanisms of nitrogen cycling in the Peru oxygen deficient zone, *Deep Sea Res., Part I*, *80*, 78–93, doi:10.1016/j.dsr.2013.05.017.
- Chronopoulou, P., F. Shelley, W. J. Pritchard, S. T. Maanoja, and M. Trimmer (2017), Origin and fate of methane in the eastern tropical North Pacific oxygen minimum zone, *ISME J.*, doi:10.1038/ismej.2017.6.
- Codispoti, L. (1995), Is the ocean losing nitrate?, *Nature*, *376*(6543), 724, doi:10.1038/376724a0.
- Codispoti, L. A., J. A. Brandes, J. P. Christensen, A. H. Devol, S. W. A. Naqvi, H. W. Paerl, and T. Yoshinari (2001), The oceanic fixed nitrogen and nitrous oxide budgets: Moving targets as we enter the anthropocene?, *Sci. Mar.*, *65*(2), 85–105, doi:10.3989/scimar.2001.65s285.
- Dalsgaard, T., B. Thamdrup, L. Fariás, and N. P. Revsbech (2012), Anammox and denitrification in the oxygen minimum zone of the eastern South Pacific, *Limnol. Oceanogr.*, *57*(5), 1331–1346, doi:10.4319/lo.2012.57.5.1331.
- Ettwig, K. F., et al. (2010), Nitrite-driven anaerobic methane oxidation by oxygenic bacteria, *Nature*, *464*(7288), 543–548, doi:10.1038/nature08883.
- Freitag, A., M. Rudert, and E. Bock (1987), Growth of *Nitrobacter* by dissimilatory nitrate reduction, *FEMS Microbiol. Lett.*, *48*, 105–109.
- Füssel, J., P. Lam, G. Lavik, M. M. Jensen, M. Holtappels, M. Gunter, and M. M. M. Kuypers (2012), Nitrite oxidation in the Namibian oxygen minimum zone, *ISME J.*, *6*, 1200–1209, doi:10.1038/ismej.2011.178.
- Ganesh, S., L. A. Bristow, M. Larsen, N. Sarode, B. Thamdrup, and F. J. Stewart (2015), Size-fraction partitioning of community gene transcription and nitrogen metabolism in a marine oxygen minimum zone, *ISME J.*, *9*(12), 2682–2696, doi:10.1038/ismej.2015.44.
- Garcia, H. E., and L. I. Gordon (1992), Oxygen solubility in seawater: Better fitting equations, *Limnol. Oceanogr.*, *37*(6), 1307–1312, doi:10.4319/lo.1992.37.6.1307.
- Gaye, B., B. Nagel, K. Dähnke, T. Rixen, and K. C. Emeis (2013), Evidence of parallel denitrification and nitrite oxidation in the ODZ of the Arabian Sea from paired stable isotopes of nitrate and nitrite, *Global Biogeochem. Cycles*, *27*, 1059–1071, doi:10.1002/2011GB004115.
- Granger, J., and D. M. Sigman (2009), Removal of nitrite with sulfamic acid for nitrate N and O isotope analysis with the denitrifier method, *Rapid Commun. Mass Spectrom.*, *23*, 3753–3762, doi:10.1002/rcm.4307.
- Gruber, N., and J. Sarmiento (1997), Global patterns of marine nitrogen fixation and denitrification, *Global Biogeochem. Cycles*, *11*, 235–266, doi:10.1029/97GB00077.
- Hamersley, M. R., et al. (2007), Anaerobic ammonium oxidation in the Peruvian oxygen minimum zone, *Limnol. Oceanogr.*, *52*(3), 923–933, doi:10.4319/lo.2007.52.3.0923.
- Haroon, M. F., S. Hu, Y. Shi, M. Imelfort, J. Keller, P. Hugenholtz, Z. Yuan, and G. W. Tyson (2013), Anaerobic oxidation of methane coupled to nitrate reduction in a novel archaeal lineage, *Nature*, *500*(7464), 567–570, doi:10.1038/nature12375.
- Horak, R. E. A., W. Qin, A. J. Schauer, E. V. Armbrust, A. E. Ingalls, J. W. Moffett, D. A. Stahl, and A. H. Devol (2013), Ammonia oxidation kinetics and temperature sensitivity of a natural marine community dominated by Archaea, *ISME J.*, *7*(10), 2023–2033, doi:10.1038/ismej.2013.75.
- Kalvelage, T., G. Lavik, P. Lam, S. Contreras, L. Arteaga, C. R. Loscher, A. Oschlies, A. Paulmier, L. Stramma, and M. M. M. Kuypers (2013), Nitrogen cycling driven by organic matter export in the South Pacific oxygen minimum zone, *Nat. Geosci.*, *6*(3), doi:10.1038/ngeo1739.
- Koch, H., S. Lückner, M. Albertsen, K. Kitzinger, C. Herbold, E. Spieck, P. H. Nielsen, M. Wagner, and H. Daims (2015), Expanded metabolic versatility of ubiquitous nitrite-oxidizing bacteria from the genus *Nitrospira*, *Proc. Natl. Acad. Sci.*, *112*(36), 11,371–11,376, doi:10.1073/pnas.1506533112.
- Lam, P., and M. M. M. Kuypers (2011), Microbial nitrogen cycling processes in oxygen minimum zones, *Annu. Rev. Mar. Sci.*, *3*(1), 317–345, doi:10.1146/annurev-marine-120709-142814.
- Lam, P., G. Lavik, M. M. Jensen, J. van de Vossenberg, M. Schmid, D. Woebken, G. Dimitri, R. Amann, M. S. M. Jetten, and M. M. M. Kuypers (2009), Revising the nitrogen cycle in the Peruvian oxygen minimum zone, *Proc. Natl. Acad. Sci.*, *106*(12), 4752–4757, doi:10.1073/pnas.0812444106.
- Levipan, H. A., V. Molina, and C. Fernandez (2014), *Nitrospina*-like bacteria are the main drivers of nitrite oxidation in the seasonal upwelling area of the eastern South Pacific (Central Chile ~36°S), *Environ. Microbiol. Rep.*, *6*(6), 565–573.
- Lipschultz, F., S. C. Wofsy, B. B. Ward, L. A. Codispoti, G. Friedrich, and J. W. Elkins (1990), Bacterial transformations of inorganic nitrogen in the oxygen-deficient waters of the eastern tropical South Pacific Ocean, *Deep Sea Res., Part A*, *37*(10), 1513–1541, doi:10.1016/0198-0149(90)90060-9.
- Lückner, S., B. Nowka, T. Rattei, E. Spieck, and H. Daims (2013), The genome of *Nitrospira gracilis* illuminates the metabolism and evolution of the major marine nitrite oxidizer, *Front. Microbiol.*, *4*, 1–19, doi:10.3389/fmicb.2013.00027.
- Lüke, C., D. R. Speth, M. A. R. Kox, L. Villanueva, and M. S. M. Jetten (2016), Metagenomic analysis of nitrogen and methane cycling in the Arabian Sea oxygen minimum zone, *Peer J.*, *4*, doi:10.7717/peerj.1924.
- Martens-Habben, W., P. M. Berube, H. Urakawa, J. R. de la Torre, and D. A. Stahl (2009), Ammonia oxidation kinetics determine niche separation of nitrifying archaea and bacteria, *Nature*, *461*(7266), 976–979, doi:10.1038/nature08465.
- McIlvin, M. R., and K. L. Casciotti (2011), Technical updates to the bacterial method for nitrate isotopic analyses, *Anal. Chem.*, *83*(5), 1850–1856, doi:10.1021/ac1028984.
- Monod, J. (1942), *Recherches sur la croissance des cultures bacteriennes*, Hermann & Cie, Paris.

- Newell, S. E., A. R. Babbín, A. Jayakumar, and B. B. Ward (2011), Ammonia oxidation rates and nitrification in the Arabian Sea, *Global Biogeochem. Cycles*, 25, GB4016, doi:10.1029/2010GB003940.
- Newell, S. E., S. E. Fawcett, and B. B. Ward (2013), Depth distribution of ammonia oxidation rates and ammonia-oxidizer community composition in the Sargasso Sea, *Limnol. Oceanogr.*, 58(4), 1491–1500, doi:10.4319/lo.2013.58.4.1491.
- Ngugi, D. K., J. Blom, R. Stepanauskas, and U. Stingl (2016), Diversification and niche adaptations of Nitrospina-like bacteria in the polyextreme interfaces of Red Sea brines, *ISME J.*, 10, 1383–1399, doi:10.1038/ismej.2015.214.
- Nowka, B., H. Daims, and E. Spieck (2015), Comparison of oxidation kinetics of nitrite-oxidizing bacteria: Nitrite availability as a key factor in niche differentiation, *Appl. Environ. Microbiol.*, 81(2), 745–753, doi:10.1128/AEM.02734-14.
- Oshiki, M., H. Satoh, and S. Okabe (2016), Ecology and physiology of anaerobic ammonium oxidizing bacteria, *Environ. Microbiol.*, 18(9), 2784–2796, doi:10.1111/1462-2920.13134.
- Padilla, C. C., et al. (2016), NC10 bacteria in marine oxygen minimum zones, *ISME J.*, 10(8), 2067–2071, doi:10.1038/ismej.2015.262.
- Peng, X., A. Jayakumar, and B. B. Ward (2013), Community composition of ammonia-oxidizing archaea from surface and anoxic depths of oceanic oxygen minimum zones, *Front. Microbiol.*, 4(177), doi:10.3389/fmicb.2013.00177.
- Peng, X., C. A. Fuchsman, A. Jayakumar, S. Oleynik, and W. Martens-habbena (2015), Ammonia and nitrite oxidation in the Eastern Tropical North Pacific, *Global Biogeochem. Cycles*, 29, 2034–2049, doi:10.1002/2015GB005278.
- Peng, X., C. A. Fuchsman, A. Jayakumar, M. J. Warner, A. H. Devol, and B. B. Ward (2016), Revisiting nitrification in the eastern tropical South Pacific: A focus on controls, *J. Geophys. Res. Ocean.*, 121, 1667–1684, doi:10.1002/2015JC011455.
- Peters, B. D., A. R. Babbín, K. A. Lettmann, C. W. Mordy, O. Ulloa, B. B. Ward, and K. L. Casciotti (2016), Vertical modeling of the nitrogen cycle in the eastern tropical South Pacific oxygen deficient zone using high-resolution concentration and isotope measurements, *Global Biogeochem. Cycles*, 30, 1661–1681, doi:10.1002/2016GB005415.
- Revsbech, N. P., L. H. Larsen, J. Gundersen, T. Dalsgaard, O. Ulloa, and B. Thamdrup (2009), Determination of ultra-low oxygen concentrations in oxygen minimum zones by the STOX sensor, *Limnol. Oceanogr. Methods*, 7, 371–381, doi:10.4319/lom.2009.7.371.
- Sigman, D. M., K. L. Casciotti, M. Andreani, C. Barford, M. Galanter, and J. K. Böhlke (2001), A bacterial method for the nitrogen isotopic analysis of nitrate in seawater and freshwater, *Anal. Chem.*, 73(17), 4145–4153, doi:10.1021/ac010088e.
- Stramma, L., G. C. Johnson, J. Sprintall, and V. Mohrholz (2008), Expanding oxygen-minimum zones in the tropical oceans, *Science*, 320(5876), 655–658, doi:10.1126/science.1153847.
- Tiano, L., E. Garcia-Robledo, T. Dalsgaard, A. H. Devol, B. B. Ward, O. Ulloa, D. E. Canfield, and N. Peter Revsbech (2014), Oxygen distribution and aerobic respiration in the north and south eastern tropical Pacific oxygen minimum zones, *Deep Sea Res., Part I*, 94, 173–183, doi:10.1016/j.dsr.2014.10.001.
- Ulloa, O., D. E. Canfield, E. F. DeLong, R. M. Letelier, and F. J. Stewart (2012), Microbial oceanography of anoxic oxygen minimum zones, *Proc. Natl. Acad. Sci.*, 109(40), 15996–16003, doi:10.1073/pnas.1205009109.
- United Nations Educational, Scientific and Cultural Organization (1994), Protocols for the Joint Global Ocean Flux Study (JGOFS) Core Measurements, I. O. Commission, United Nations Educational, Scientific and Cultural Organization.
- Ushiki, N., M. Jinno, H. Fujitani, T. Suenaga, A. Terada, and S. Tsuneda (2017), Nitrite oxidation kinetics of two *Nitrospira* strains: The quest for competition and ecological niche differentiation, *J. Biosci. Bioeng.*, 123(5), 581–589, doi:10.1016/j.jbiosc.2016.12.016.
- Ward, B. B., A. H. Devol, J. J. Rich, B. X. Chang, S. E. Bulow, H. Naik, A. Pratihary, and A. Jayakumar (2009), Denitrification as the dominant nitrogen loss process in the Arabian Sea, *Nature*, 461(7260), 78–81, doi:10.1038/nature08276.
- Weigand, M. A., J. Foriel, B. Barnett, S. Oleynik, and D. M. Sigman (2016), Updates to instrumentation and protocols for isotopic analysis of nitrate by the denitrifier method, *Rapid Commun. Mass Spectrom.*, 30(12), 1365–1383, doi:10.1002/rcm.7570.